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ANALYSIS OF VOLATILE ORGANIC COMPOUND OF MALLAWA HONEY

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ABSTRACT

This study aimed to determine the volatile organic compound for Mallawa honey. The quality of honey is determined from the nectar source, geographic location, and the processing technology. Volatile organic compound is one of the indicators in honey to determine the quality marker and this compound is formation aroma of honey.This research was done in three stages, namely the stage of sample preparation, extraction phase, and phase identification. Stages of sample preparation was performed to determine sampling locations, the determination of the location of the extraction and identification. Extraction is done in analytical chemistry laboratory Science Faculty, Hasanuddin University. Honey samples were extracted with n-heksan. The identification process carried out in an integrated laboratory Department of Chemical Science Faculty of Hasanuddin University, GCMS performed while the data in Forensik laboratory, POLRI. The results were obtained 35 volatile organic compounds, which are grouped into six classes of compounds, namely: hydrocarbons, acid, aromatic, ketones, aldehides and the other compounds.

Keyword : Mallawa Honey, volatile organic compound, GCMS

1. INTRODUCTION

Honey is a natural sweetener produced by bees with the nectar of flowers raw materials. Two factors needed to produce honey. First, flower nectar is a raw material for making honey. Second, the insects are bees that are energy experts. Honey is a natural saturated sugar solution, which mainly consists of a mixture of complex carbohydrates. It also contains water and minor components but contains important nutrients such as vitamins, minerals, enzymes, organic compounds, free amino acids and various volatile compounds. However, these minor components responsible for organoleptic and nutritional properties of honey.[2] One of the important components in honey are volatile compounds which are compounds that are volatile because it has a low molecular weight. Volatile compounds are the main factors responsible for the aroma, so it is one of the most important features to detect counterfeit products. Aroma honey is very complex because it involves dozens of volatile compounds.[2]

Several volatile compounds derived directly from the flower (nectar) are visited by bees, therefore, based on the original scent of flowers (nectar). Honey inherit properties of plants, color, aroma, flavor, density, physical and chemical properties, (depending on the flowers sucked by the bees) despite the weather conditions also influence the composition and properties of honey.[11] The amount of volatile elements were already found in honey are about 500 species, including the orange honey is metilantranilat, lilak aldehydes, hotrienol and 1-p-menthen-al and in honey eucalyptus them nonanol,

ISSN 1411-2132
Volume 17 No. 2

nonanal, nonanoat, and isofron (3.5 , 5-trimethyl-2-enone cyclohexane). There are 4 new volatile elements that are found in honey which is 1.3-propanodiol, 2-methyl butanoic acid, 3,4-dimethyl-3-heksen-2-one and 6-methyl-5-okten-2-on.[4] Volatile compounds are also found in local honey, especially in South Sulawesi who have been identified as many as 21-27 kinds of volatile compounds, which are grouped into the class of hydrocarbons, ketones, acids, alcohols, amines, aldehydes and oxime. [11][1]

Volatile compounds in each of honey depends on the origin of flowers, the composition of the nectar which is inhaled by the bees and their geographic origin so as to provide profiles of different scents of honey, and is a characteristic that can be used to distinguish honey based on the origins of botany them[4] , Geographical location Mallawa topographical districts that are in the ground slope between 3-70% and the altitude above sea level between 15-458 M. stretch of rocks making up includes karst hills, volcanic mountains, hills intrusion and sediments [2]. Rock constituent dominated by karst hills composed of limestone is one sign that the area was once a sea [3]. Spermonde Islands are located around the Makassar Strait is thought to have a correlation with the geographical conditions Mallawa, thus indirectly may affect the presence of minerals in the area Mallawa. Research on volatile compounds at various examples of honey that is based on regional origin or country in the world already exists, but the honey specifically from districts Mallawa Maros has not been studied compounds volatilnya nor the content of volatile compounds in nectar plants that are in the forest Mallawa ,

Theory

Honey is a unique product of an insect that contains a high percentage of carbohydrates, practically no protein or fat. The nutritional value of honey depends on the content of simple sugars, fructose and glucose. The sweet foodstuffs are thick with gold until dark, produced in the honey sacs of various kinds of wasps and from a variety of flower nectar. Flavor and fragrance is influenced by the type of flower where the nectar is collected.

Volatile Organic Compounds In Honey

One of the important components in honey are volatile compounds. Volatile compounds are compounds that are volatile in honey due to low molecular weight. Volatile compounds are the main factors responsible for the aroma as it can evaluate the organoleptic and authenticity of foodstuffs.[2] Factors affecting honey aroma is a volatile element in it especially gluconic acid and proline.[10] Volatile Elements of each different plant nectar that was typical aroma arising or different. The amount of volatile elements were already found in about 500 types of honey, including honey orange is metilantranilat, lilak aldehydes, hotrienol and 1-p-menthen-al and in honey eucalyptus them nonanol, nonanal, nonanoat, and isofron (3.5, 5- trimetilsikloheksan-2-enone). There are 4 new volatile elements that are found in honey which is 1.3-propanodiol, 2-methyl butanoic acid, 3,4-dimethyl-3-heksen-2-one and 6-methyl-5-okten-2-on [4][12] considers the taste of honey is caused by sugars, gluconic acid and proline, whereas the specific taste of honey with innumerable variations cause the taste as by various glucosides and alkaloids that are typical for the plant source of nectar. With growing gas panggunaan Liquid

ISSN 1411-2132
Volume 17 No. 2

Chromatography, increasingly, studies on compounds of volatile acids (Volatile Acids) that there are various sources of honey, honey aroma forming many compounds, such as formaldehyde, acetaldehyde, acetone, isobutilraldehid and diacetyl (Table 3). Aroma striking the honey comes from nectar orange citrum caused by Methyl anthranilat that although there are only a few.[9]

Table 1. Formation of Aroma Honey In Honey

carbonyl	Alcohol	Esther
acetaldehyde	Benzylalkohol	Diethyl Ether
acetone	B-METALLY	ethyl format
Butiraldehido	alcohol	methyl format
Formaldehyde	ethanol	
Isovaleraldehido	isobutanol	
Metaklorein	2-butanol	
Methyl ethyl ketone	2-methyl-1-butanol	
	3-methyl-1-butanol	
Propionaldehido	3-methyl-2-butanol	
	3-pentanol	
	n-butanol	
	n-pentanol	
	n-propanol	
	Pheniletil alcohol	

Sumber : [9]

2. METHODS

Materials and tools

The materials used are honey Mallawa, n-heksane, ethyl acetate, aquabidesh, Na_2SO_4.The tools used in this study include GCMS models Agilent 5975C, a beaker of 100 ml, a measuring cup 10 ml, pipette 1 ml, vortex, sonicator, analytical balance, mixers, centrifuges, bottle vial closed 10 ml brown bottle 30 ml and laboratory equipment commonly used.

Time and Place Research

The research was conducted from April 2015 to June 2015.Penelitian Rdiasi conducted at the Chemical Laboratory and the University of Hasanuddin Makassar Police Forensic Laboratory.

Sample preparation

Sampling was carried out in the forest honey Mallawa Kab. Maros South Sulawesi with five different points. Honey samples stored in glass bottles cool and dry. First of all honey weighed (1g) and diluted with 1 mL aquabidest. Divortex mixture for 2-3 minutes to ensure mixing honey and aquabidest before the liquid-liquid extraction followed by identification of compounds using GCMS.

Liquid-liquid extraction

2 different organic solvents with increasing polarity selected: 1) n-hexane, 2) ethyl acetate. Extraction continues to run on a single sample. First of all, n-hexan (4 mL) was added to the sealed vial containing honey that has been diluted. Then, the mixture was divortex at 1500

ISSN 1411-2132
Volume 17 No. 2

rpm for 2 minutes before disonikator for 20 minutes to separate the organic layer from the aqueous layer. The top layer containing the organic solvent was transferred to a closed vial while the bottom layer coupled with the same solvent and then the above method is repeated up to three times. ekstraksi.lapisan organic extraction results first, second and third combined and dicentrifuge at 2500 rpm for 10 minutes and the upper layer pipette. Then added with Na_2SO_4 to remove the remaining water and ready to be analyzed using GCMS.

Analysis by GC-MS

GCMS analysis was performed on Agilent HP-5ms 5975c.Kolom fused silica capillary column, and runs at a constant pressure helium is used as a carrier gas. The following conditions are used: initial temperature of 40 °C, equilibration time of 5 minutes, the temperature gradient of 25 °C / min, a final temperature of 310 °C.

3. RESULTS AND DISCUSSION

Overall obtained 58 kinds of volatile compounds from the five samples of honey derived from forests Mallawa using GCMS. A total of 35 volatile compounds obtained from the honey extraction using non-polar solvent (n-hexane). Previous studies have identified volatile compounds at different honey such as honey Palestinians successfully gained as much as 30 volatile compounds.[8] Honey wanderer Malaysia, has been identified as 34 volatile compounds (Syazana, 2013), as well as honey from the northern area of the province of Nuble Chile that produce a further 34 volatile compound.[4] According to [7] using 3 kinds of different solvents are n-hexane, ethyl acetate and chloroform result 32 volatile compounds were classified as hydrocarbons, acids, aldehydes, ketones, derivatives of benzene, derivatives of terpenes, alcohols, derivatives furans, and other Pyrans (Tiopen, metamin hydrochloride, N-methyl-D3-Arizidin, propannitril).

In a sample of honey Mallawa taken from five different points, obtained the volatile compounds that use a fraction of n-hexane with the classification of hydrocarbons such as alkanes and aldehyde (Tetradekana, 1-hexadecane, hexadecane, 1-Oktadekana, Oktadekana, Eikosana, 1-Dekosana, Tetrakosana, Pentakosana, Heksakosana, Heptakosana, Oktakosana, Heptadekana, dodecane, Heptasiloksana, E-15 Heptadekanal 1-Oktadekanal, Trikosana, Heneikosana), phenol (2,6-bis (1,1-dimetiletil)-4-methyl,), acid (Pentaflouropionik acid, acid Dehidroabietik) aldehyde (3,4,6,8-Tetrametilazulena-1-karbaldehida, Nafto (2,3-B) Furan-3-Karboksildehida), toluene (Butilat Hidroksitoluen), Aromatic (2,6-Diisopropilnaftalena, Benzene, 2-(Butenyl)-5-(1,1-Dimetiletil)-1,3-Dimetil-,1,3-Diformil-2-kloro-5-isopropilbenzen,2-metil-4-metoksi-1,1-befenil naphthalene, 1-methoxy-8- (1-methyl-ethenyl),) and others (3-Phenyl-5- (T-butyl) Piridazin, 2,4,8-Meteno-3A, 8C-Diazapentalino, Oksazol).

Honey volatile compounds obtained from five different points either using n-hexane fraction chromatograms are shown in the following:

International Journal
Marina Chimica Acta
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ISSN 1411-2132
Volume 17 No. 2

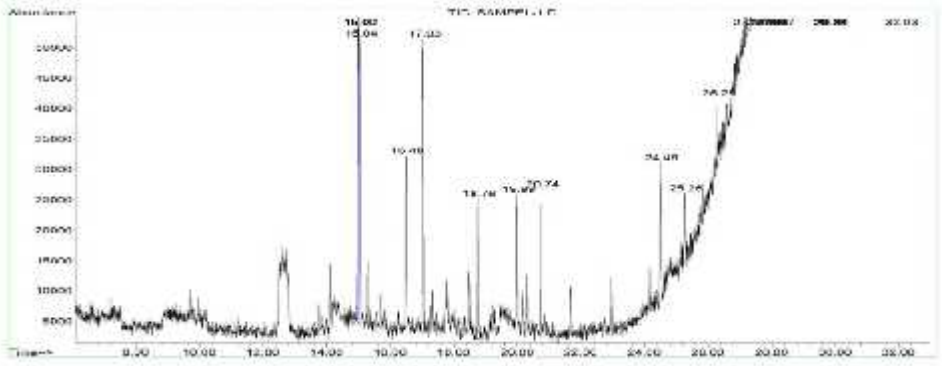

Figure 1. Sample 1 chromatograms with solvent n-hexane

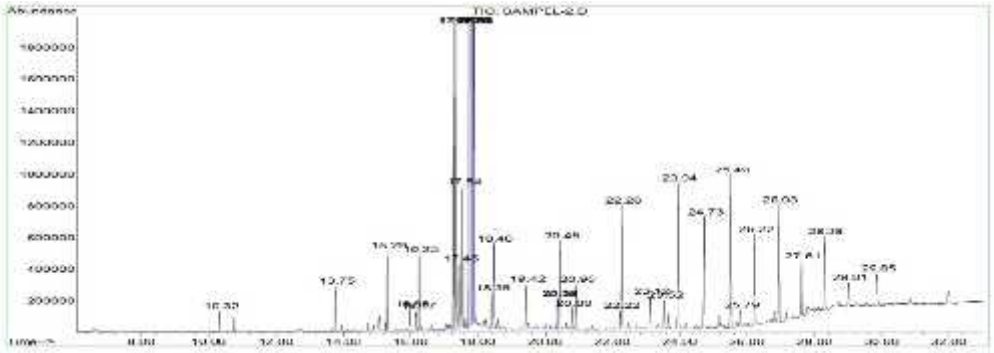

Figure 2. Sample 2 chromatograms with solvent n-hexane

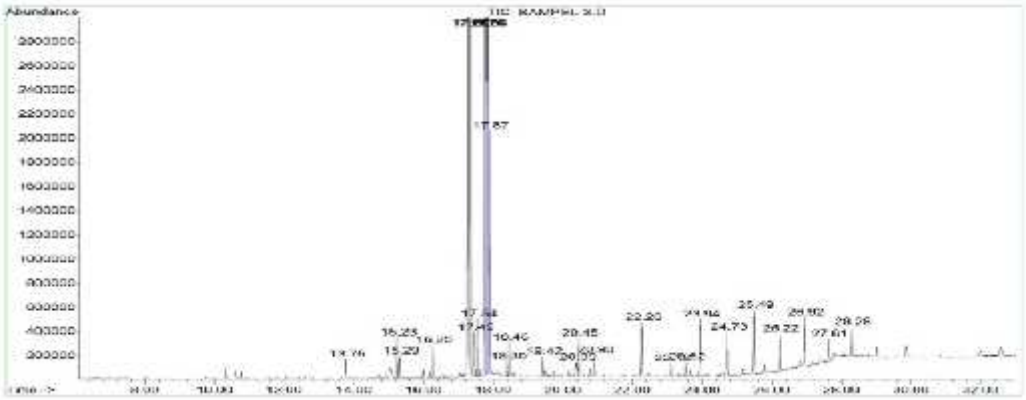

Figure 3. Chromatogram Sample 3 with solvent n-hexane

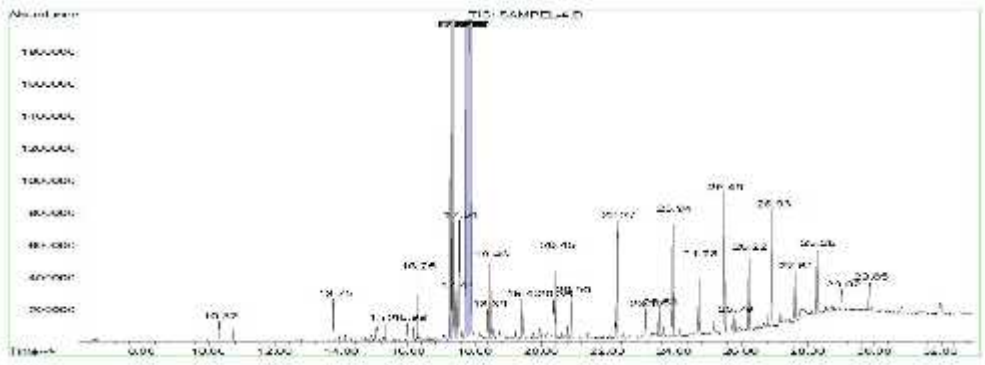

Figure 4. Chromatogram Sample 4 with solvent n-hexane

International Journal
Marina Chimica Acta
The University of Hasanuddin

ISSN 1411-2132
Volume 17 No. 2

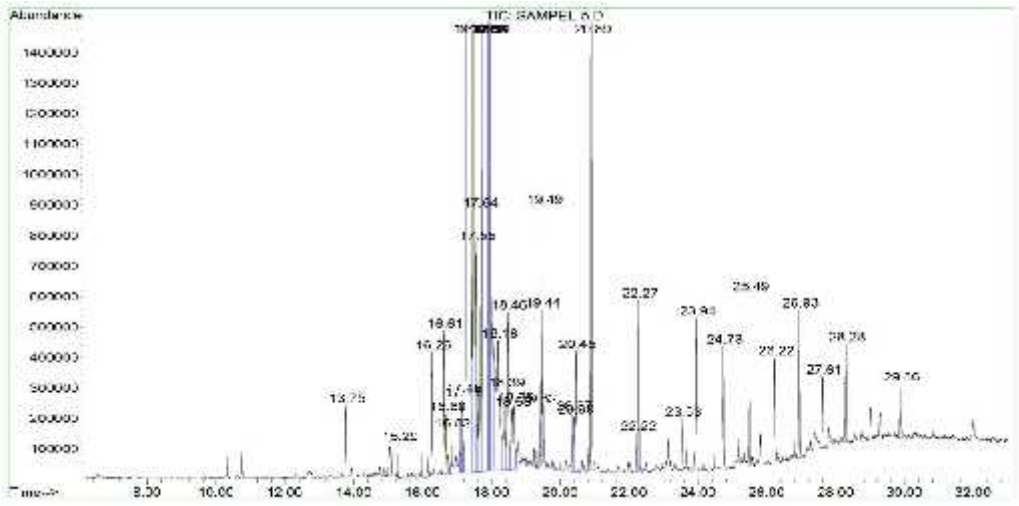

Figure 5. Sample chromatograms 5 with solvent n-hexane

Based on test results, showing that not all honey samples 1,2,3,4 and 5 are voletil same compound, but there are some volatile compounds are virtually owned by either the honey samples of honey samples of honey samples 2,3,4 and 5 for example, is a class of compounds alkanes such as: Tetradekana, Oktadekana, Eikosana, hexadecane. While the volatile compounds 2,6-Diisopropilnaftalena, Heptakosana, Phenol, 2,6-bis (1,1-dimetiletil) -4-methyl, Heneikosana, Heksakosana, dodecane only owned by three types of honey samples. This proves that nearly all volatile compounds derived from honey samples 2,3,4 and 5 are the same class of compounds, while the honey samples 1 to the type of solvent n-hexane was not detected because the percentage of similarity of compounds that are in the range of 70% down temporarily which to sample honey 2,3,4 and 5 percentage similarity of compounds within the range of 90 % and above.

Table 2. Volatile Compounds honey and nectar were identified using n-hexane fraction

No	Nama Senyawa	M1	M2	M3	M4	M5
1	Tetradekana	-	√	√	√	√
2	Butilat Hidroksitoluen	-	√	-	-	-
3	1-Heksadekana	-	√	-	√	-
4	Heksadekana	-	√	√	√	√
5	2,6-Diisopropilnaftalena	-	√	√	√	√
6	1-Oktadekana	-	√	-	√	-
7	Oktadekana	-	√	√	√	√
8	Eikosana	-	√	√	√	√
9	1-Dekosana	-	√	-	√	-
10	Tetrakosana	-	√	-	√	√
11	Pentakosana	-	√	-	√	√
12	Heksakosana	-	√	√	√	√
13	Heptakosana	-	√	-	√	√
14	Oktakosana	-	√	√	√	√
15	Heptadekana	-	√	-	√	√
16	Dodekana	-	√	√	√	√
17	Fenol, 2,4-bis (1,1-dimetiletil)	-	√	-	-	√
18	Fenol, 2,6-bis (1,1-dimetiletil) -4-metil	-	√	-	√	√

ISSN 1411-2132
Volume 17 No. 2

19	Asam Pentaflouropionik	-	-	√	√	-
20	3-Fenil-5-(T-butil) Piridazin	--	-	-	√	-
21	Sikloheptasiloksana	-	-	-	√	-
22	Heptasiloksana	-	-	-	√	-
23	E-15 Heptadekanal 1-Oktadekanal	-	-	-	√	-
24	Naftol (2,1-B) Furan	-	-	-	√	-
25	Asam Dehidroabietic (asam 1-fenantrenkarboksilik)	-	-	-	√	-
26	Benzene, 2-(Butenyl)-5-(1,1-Dimetiletil)-1,3-Dimetil-	-	-	-	√	-
27	2-metil-4-metoksi-1,1-befenil naftalena, 1-metoksi-8-(1-metil-etenil)	-	-	-	√	-
28	1,3-Diformil-2-kloro-5-isopropilbenzen	-	-	-	√	-
29	3,4,6,8-Tetrametilazulena-1-karbaldehida	-	-	-	√	-
30	Nafto (2,3-B) Furan-3-Karboksildehida	-	-	-	√	-
31	2,4,8-Meteno-3A, 8C-Diazapentalino	-	-	-	√	-
32	Oksazole, 2,5-Difenil-	-	-	-	√	-
33	Trikosana	-	-	-	-	√
34	Heneikosana	-	-	-	-	√
35	2,6,10,14,18,22-Tetrakosaheksana-	-	-	-	-	√

Source: Primary Data 2015

In Table 2 explained that the volatile compounds derived from honey by using a fraction of n-hexane is dominated by volatile compounds from the class of hydrocarbons such as (1) Tetradekana which is also found in honey Asphodel (Jercovic, 2011) and honey Acer (Jercovic, 2011), (2) hexadecane, (3) Heksakosana, (4) Oktadekana, (5) Eikosana which is also found in honey Thailand (A.andreniformis, A. Cerana, A.dorsata, A.florea and A.mellifera (Jercovic, 2010), (6) Tetrakosana, (7) Oktakosana, (8) Heptakosana, (9) Heptadekana which is also found in honey Thyme (Mannas, 2007) and honey Asphodel (Jercovic, 2011) and (10) dodecane were also found in honey Asphodel (Jercovic, 2011) and in honey Acer (Jercovic, 2010). the rest is a class of organic acids and acid Dehidroabietik Pentaflouropionik acid and phenolic groups such as Phenol, 2,4-bis (1,1-dimetiletil) and Phenol, 2,6 -bis (1,1-dimetiletil) -4-methyl.

4. CONCLUSION

Based on research data obtained 21 volatile organic compounds of honey Mallawa and have been identified into several groups, namely hydrocarbons (28%), acid (9%), amine (11%), alcohol (18%), aldehydes (4%), siloxane (22%), and ketone (8%).

REFERENCES

[1] Astuti, 2014. *Kajian Senyawa Volatil Madu Trigona Sulawesi selatan sebagai Antimikroba.* Tesis tidak diterbitkan. Makassar. Program Pascasarjana Fakultas Kesehatan Masyarakat Universitas Hasanuddin

[2] Baroni, M. V., Nores, M. L., Diaz, M., Chiabrando, G. A., Fassano, J.P., Costa, C and Wunderlin, D., 2006. Determination of Volatile Organic Compound petterns charactheristic of five univoral honeys by *solid-phase microextraction-Gas chromatography-mass spectrometry*

ISSN 1411-2132
Volume 17 No. 2

coupled to chemimetrics. *J. Agric. Food Chem. 54: 7235-7241.*

[4] Barra, M. P. G., Ponce-Diaz., Venegas-Gallegos, C, 2010. Volatile Compounds In Honey Produced In The Central Valley *Of Nuble Province*, Chile. *Chilean J. Agric Res 70(1): 75-84 (January-March 2014)*

[5] Jerkovic, 2010. Volatiles from a Rare Acer spp. Honey Sample from Croatia. Molecules 2010, 15, (online) 4572-4582;doi:3390/molecules15074572. ISSN 1420-3049. Diakses pada tanggal 10 Maret 2014

[6] Jerkovic, 2011. Volatiles from a Rare Acer spp. Honey Sample from Croatia. Molecules 2010, 15, (online) 4572-4582;doi:3390/molecules15074572. ISSN 1420-3049. Diakses pada tanggal 10 oktober 2014

[7] Manyi, C. E., Clarke, A. M., and Roland., 2011. Identification of Volatile Compounds in Solvent Extracts of Honeys Produced in South Africa. *Afr. J. Agric. Res., 6(18): 4327-4334.*

[8] Odeh *et al,* 2007. A Variety Of Volatile Compounds As Markers In Palestinian Honey from Thymus Capitatus, Thymelaeae Hirsuta, And Tolpis Virgata. Chemistry 101 (2007) 1393-1397

[9] Sihombing, 1994. *Ilmu Ternak Lebah Madu*. Yogyakarta : Gajah Mada Universitas Press.

[10] Suarez, 2010. Contribution Of Honey In Nutrition And Human Health: A Review. *Mediterr J. Nutr. Metab (2010) 3:15-23.*

[11] Zakaria, 2013. *Analisis Senyawa Organik Volatil Pada Madu Mallawa*. Laporan Akhir Penelitian. STAIN Watampone. Mallawa

[12] Maeda *et al*, 1962. *Application of near-infrared spectroscopy in quality assessment of beehoney*. University of Hong kong